Distal renal tubular acidosis with multiorgan autoimmunity: A case report

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Abstract: A 61-year-old woman with a history of pernicious anemia presented with progressive muscle weakness and dysarthria. Hypokalemic paralysis (serum potassium, 1.4 mEq/L) due to distal renal tubular acidosis (dRTA) was diagnosed. After excluding several possible causes, dRTA was considered autoimmune. However, the patient did not meet criteria for any of the autoimmune disorders classically associated with dRTA. She had very high antibody titers against parietal cells, intrinsic factor, and thyroid peroxidase (despite normal thyroid function). The patient consented to a kidney biopsy, and acid-base transporters, anion exchanger type 1 (AE1), and pendrin were undetectable by immunofluorescence. Indirect immunofluorescence detected diminished abundance of AE1- and pendrin-expressing intercalated cells in the kidney, as well as staining by the patient's serum of normal human intercalated cells and parietal cells expressing the adenosine triphosphatase hydrogen/potassium pump (H(+)/K(+)-ATPase) in normal human gastric mucosa. The dRTA likely is caused by circulating autoantibodies against intercalated cells, with possible cross-reactivity against structures containing gastric H(+)/K(+)-ATPase. This case demonstrates that in patients with dRTA without a classic autoimmune disorder, autoimmunity may still be the underlying cause. The mechanisms involved in autoantibody development and how dRTA can be caused by highly specific autoantibodies against intercalated cells have yet to be determined.

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Distal renal tubular acidosis with multi-organ autoimmunity, a case report.

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Abstract

A 61-year old woman with a previous history of pernicious anemia presented with progressive muscle weakness and dysarthria. She was diagnosed with hypokalemic paralysis (serum potassium 1.4 mEq/L) due to distal renal tubular acidosis (dRTA). After excluding several possible causes, dRTA was considered autoimmune. However, the patient did not meet the criteria for any of the autoimmune disorders classically associated with dRTA. She did have very high antibody titers against parietal cells, intrinsic factor, and thyroid peroxidase (but with normal thyroid function). The patient consented to a kidney biopsy. Immunofluorescence studies revealed the absence of the acid-base transporters anion exchanger type 1 (AE1) and pendrin. Indirect immunofluorescence studies demonstrated (1) diminished abundance of AE1 and pendrin expressing intercalated cells in her kidney, and positive staining by her serum of (2) normal human intercalated cells and (3) parietal cells expressing the H^+/K^+-ATPase pump in normal human gastric mucosa. The dTRA is likely caused by circulating autoantibodies against intercalated cells (with possible crossreactivity against structures containing the gastric H^+/K^+-ATPase pump).

This case demonstrates that in patients with dRTA without a classical autoimmune disorder, autoimmunity may still be the underlying cause. The mechanisms why autoantibodies are developed and how highly specific autoantibodies against intercalated cells can cause dRTA remains to be uncovered.

Introduction

Distal renal tubular acidosis (dRTA, also called type I RTA) is caused by an inability of the kidney collecting duct to acidify urine (1). The metabolic acidosis in dRTA is characterized by a normal anion gap, urinary pH > 5.3, and low ammonium excretion. dRTA is usually caused by a proton secretion defect in the intercalated cells. This proton secretion defect explains why dRTA is usually accompanied by hypokalemia, because it indirectly causes renal potassium losses and cellular shifts of potassium. dRTA may be inherited or acquired by drugs, hypercalciuria, or an underlying autoimmune disorder (2). It has been reported in various autoimmune disorders including Sjögren's syndrome, primary biliary cirrhosis, autoimmune hepatitis, systemic lupus erythematosus, and rheumatoid arthritis (2). How these autoimmune disorders cause dRTA is not well known. dRTA is a relatively common complication of Sjögren's syndrome, and studies have suggested the presence of autoantibodies against acid-base transporters (3-5). Here, we report a case of dRTA associated with circulating antibodies against intercalated cells. Surprisingly, the patient did not have one of the systemic autoimmune disorders associated with dRTA, but did have multiorgan autoimmunity involving the kidney, stomach and thyroid glands.

Case report

A 61-year old woman presented with progressive muscle weakness and dysarthria since one week. Her dietary habits were unremarkable and she did not have diarrhea. She used no other medication than folic acid (5 mg/day) and vitamin B12 injections because of pernicious anemia (her only medical history). She did not use H2-receptor antagonists or proton pump inhibitors. Besides paralysis (muscle strength 2/5), physical examination was normal, including examination of the thyroid. Laboratory tests at admission revealed severe hypokalemia (serum potassium 1.4 mEq/L) and non-anion gap metabolic acidosis (serum pH 7.14, bicarbonate 9 mEq/l). Hypokalemia was due to renal potassium losses (urine potassium 20 mEq/l, fractional potassium excretion 21%). The metabolic acidosis was characterized by an inappropriately high urine pH of 7.1 and a positive urine anion gap (+21 mEq/l), suggesting renal tubular acidosis (RTA). Other laboratory tests were normal, including thyroid and kidney function. She had normal sized kidneys without nephrocalcinosis. The patient was hospitalized for several weeks, initially at the intensive care unit for intravenous potassium and bicarbonate supplementation under telemetry, and is currently still treated with oral sodium bicarbonate (1500 mg daily) and potassium citrate (1800 mg daily) maintaining a normal serum potassium (4.1 mEq/L) and bicarbonate (25 mEq/L). She is currently followed-up as outpatient for electrolyte and acid-base balance, and gastric and thyroid function.

After the acute phase, additional tests were performed to investigate the type and cause of RTA. An acidification test using fludrocortisone and furosemide was performed during which she failed to reach a urine pH < 5.3 (lowest urine pH 5.5) (6). In addition, renal ammonium excretion was assessed by calculating the urine osmolal gap and found to be very low in view of the existing acidosis (11 mEq/l). These findings confirmed dRTA, although a fractional bicarbonate excretion test was not performed to formally exclude a proximal RTA. The

diagnosis was further supported by the absence of other markers of proximal tubular dysfunction (no hypouricemia, hypophosphatemia, proteinuria or glucosuria), and the absence of paraprotein and urinary light chains. Although no previous measurements of serum potassium or bicarbonate were available, a hereditary form of dRTA seemed unlikely (negative family history, late age of presentation). Repeated history and physical examination did not provide clues for a systemic autoimmune disorder. There was no hypercalciuria. Antinuclear antibodies, anti-neutrophil cytoplasmic antibodies and rheumatoid factor tested negative, as did antibodies against SS-A, SS-B, and tissue transglutaminase. Antibodies against parietal cells, intrinsic factor (titers not available), and thyroid peroxidase (titer >1300 IU/mL, normal range < 60 IU/mL), however, were strongly positive.

With informed consent of the patient, a kidney biopsy was performed. Light microscopy showed normal glomeruli and an atrophic aspect of some of the proximal tubuli without any other histological changes (data not shown). Subsequently, we performed indirect immunofluoresence on the patient's kidney tissue and with the patient's serum on normal human kidney and stomach tissue. In the indirect immunofluorescence studies we tested aquaporin-2 (AQP2) as marker of principal cells and three acid-base transporters in intercalated cells, including the a4 and B1 subunits of the proton pump (H^+-ATPase), the anion exchanger 1 (AE1, a marker for acid-secretory type A intercalated cells), and pendrin (a marker for bicarbonate-secretory type B intercalated cells). Remarkably, staining for AE1 and pendrin was absent in the patient's kidney (**Figure 1**). Although residual staining for the a4 and B1 subunits was present, these co-localized in the same cells with AQP2 (**Figure 1**).

The incubation with the patient's serum on normal human kidney caused strong staining exclusively in both types of intercalated cells, whereas this was not present with control sera

from 5 persons (male and female, age between 30-60 years) (**Figure 2**). To confirm that the staining occurred in intercalated cells, we performed double labelling with AE1 (green) and AQP2 (white, **Figure 2**). These studies showed that the patient's serum stained type A intercalated cells because of co-localization with AE1. Because the patient's serum also stained cells that were negative for AE1 and AQP2, we believe this additional staining occurred in type B intercalated cells. In gastric tissue, staining for the α-subunit of H^+/K^+-ATPase, a marker of acid-secretory parietal cells in the gastric mucosa, revealed a strong overlay with the signal from the patient's serum (green) and the α-subunit of the H^+/K^+-ATPase (red) (**Figure 2**). The staining appeared to have partial but substantial overlap, suggesting that the pump itself or associated proteins are part of the antigen. With control serum there was no staining for cells expressing the α-subunit of the H^+/K^+-ATPase (**Figure 2**).

Discussion

We report an unusual case of dRTA, which was clearly of autoimmune origin, but could not be linked to any systemic autoimmune disorder known to be associated with dRTA. By performing indirect immunofluorescence studies we demonstrate (1) diminished abundance of AE1 and pendrin expressing intercalated cells in the kidney of the patient, and positive staining by the patient's serum of (2) normal human intercalated cells and (3) parietal cells expressing the H^+/K^+-ATPase pump in normal human gastric mucosa. We conclude that dTRA is likely caused by circulating autoantibodies against intercalated cells with possible crossreactivity against structures containing the gastric H^+/K^+-ATPase pump. The co-localization of H^+-ATPase with AQP2 may suggest that intercalated cells have either completely disappeared and that principal cells express some markers of intercalated cells or that the differentiation process is affected by autoantibodies and that cells coexpressing H^+-ATPases and AQP2 represent an intermediate cell type as has been reported in mice lacking the transcription factor Foxi1 which is required for normal intercalated cell differentiation (7). It is unclear if one common epitope is present in kidney, stomach, and possibly thyroid, or whether multiple autoantibodies target different epitopes in these three tissues. The B1 subunit of H^+-ATPase is not expressed in stomach or thyroid glands whereas the ubiquitious B2 subunit is present in every tissue and cell type. Also, pendrin and AE1 are absent from stomach. In addition, cross-reactivity of the patient's serum with red blood cells would be expected in the case of AE1 reacting autoantibodies (4). Moreover, patients with mutations in AE1 or H^+-ATPase subunits are not known to have thyroid or gastric disorders (8). Autoantibodies against pendrin have been reported in patients with auto-immune thyroiditis and hypothyroidism (9). Patients with mutations in pendrin present with deafness that can be accompanied by goiter and hypothyroidism; electrolyte and acid-base disorders have only been reported in these patients in the context of thiazide use or inter-current illness (9-11).

Also, the pathogenesis of dRTA is difficult to reconcile with dysfunctional pendrin, because this transporter is involved in renal bicarbonate secretion and sodium chloride reabsorption. Another candidate protein would be SLC26A7, although we did not analyze this transporter in our immunolabeling studies. SLC26A7 is expressed in intercalated cells and stomach and mice lacking SLC26A7 suffer from RTA and reduced gastric acid secretion (12). Of interest, the constellation of autoantibodies against parietal cells, intrinsic factor, and thyroid peroxidase and the occurrence of dRTA has been reported previously (13). In this series of 113 patients, a complement fixing autoantibody was identified that reacted with the renal collecting duct.

In conclusion, this unusual case demonstrates that in patients with dRTA, autoimmune disorders must be considered that do not fit the classic disorders associated with dRTA. The diagnosis can be made by the combination of excluding these classic syndromes and the demonstration of autoantibodies against affected organs. The exact mechanisms why autoantibodies are developed and how highly specific autoantibodies against intercalated cells can cause dRTA remains to be uncovered.

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Figure 1: Loss of intercalated cells expressing AE1 and pendrin in the patient's kidney

(A) Healthy human kidney was stained for AE1 (green) and the B1 H^+-ATPase subunit, and cell nuclei (blue). **(B)** The patient's kidney shows no staining for AE1 in collecting ducts (*) but in red blood cells (arrow). **(C)** Healthy human kidney stained for pendrin (green), AQP2 (white), the a4 H^+-ATPase subunit (red), and cell nuclei (blue). Pendrin colocalizes in some cells with the a4 H^+-ATPase subunit (arrow, yellow overlay). **(D)** Staining of patients kidney for AE1 (red), AQP2 (white), and pendrin (green). Nuclei are stained in blue. No staining for pendrin was detectable in the patient's kidney but all cells in the collecting duct (*) were positive for AQP2. **(E,F,G)** Patient's kidney stained for the B1 H^+-ATPase subunit (red, E), and AQP2 (white, F), panel G depicts the overlay of all channels. All cells are positive for AQP2 but some cells have also a weak but distinct luminal red staining suggesting colocalization of AQP2 and B1 in the same cells. Original magnification 400-630 x.

Figure 2: Autoantibodies against renal intercalated cells and gastric parietal cells

(A) Incubation of healthy human kidney with serum from a control person (red, 1:1000) and staining of type A intercalated cells with antibodies against AE1 (green). No specific red staining detectable. **(B)** Incubation of healthy human kidney with patient's serum (red, 1:1000) and antibodies against AE1 (green) demonstrating specific staining of type A intercalated cells. **(C)** Incubation of healthy human kidney with patient's serum (red) and antibodies against AE1 (green) and AQP2 (white) demonstrates staining with autoantibodies of cells negative for AQP2 and positive (asterisk: type A intercalated cells) or negative for AE1(arrow: type B intercalated cells). **(D)** Incubation of healthy human stomach with patient's serum (red) and antibodies against gastric α-H^+/K^+-ATPase (green), a marker of acid-secretory parietal cells, showing partial colocalization of both signals indicated by the

yellow color. Insert with higher magnification of parietal cells (1000x magnification). Original magnification 400 x.

References

1. Unwin RJ, Shirley DG, Capasso G. Urinary acidification and distal renal tubular acidosis. *J Nephrol*. 2002;15 Suppl 5:S142-50.

2. Rodriguez Soriano J. Renal tubular acidosis: the clinical entity. *J Am Soc Nephrol.* 2002;13(8):2160-70.

3. Cohen EP, Bastani B, Cohen MR, Kolner S, Hemken P, Gluck SL. Absence of H(+)-ATPase in cortical collecting tubules of a patient with Sjogren's syndrome and distal renal tubular acidosis. *J Am Soc Nephrol*. 1992;3(2):264-71.

4. Walsh S, Turner CM, Toye A, Wagner C, Jaeger P, Laing C, et al. Immunohistochemical comparison of a case of inherited distal renal tubular acidosis (with a unique AE1 mutation) with an acquired case secondary to autoimmune disease. *Nephrol Dial Transplant.* 2007;22(3):807-12.

5. Han JS, Kim GH, Kim J, Jeon US, Joo KW, Na KY, et al. Secretory-defect distal renal tubular acidosis is associated with transporter defect in H(+)-ATPase and anion exchanger-1. *J Am Soc Nephrol*. 2002;13(6):1425-32.

6. Walsh SB, Shirley DG, Wrong OM, Unwin RJ. Urinary acidification assessed by simultaneous furosemide and fludrocortisone treatment: an alternative to ammonium chloride. *Kidney Int*. 2007;71(12):1310-6.

7. Blomqvist SR, Vidarsson H, Fitzgerald S, Johansson BR, Ollerstam A, Brown R, et al. Distal renal tubular acidosis in mice that lack the forkhead transcription factor Foxi1. *J Clin Invest*. 2004;113(11):1560-70.

8. Batlle D, Haque SK. Genetic causes and mechanisms of distal renal tubular acidosis. *Nephrol Dial Transplant*. 2012;27(10):3691-704.

9. Kandasamy N, Fugazzola L, Evans M, Chatterjee K, Karet F. Life-threatening metabolic alkalosis in Pendred syndrome. *Eur J Endocrinol*. 2011;165(1):167-70.

10. Wagner CA, Mohebbi N, Capasso G, Geibel JP. The anion exchanger pendrin (SLC26A4) and renal acid-base homeostasis. *Cell Physiol Biochem*. 2011;28(3):497-504.

11. Pela I, Bigozzi M, Bianchi B. Profound hypokalemia and hypochloremic metabolic alkalosis during thiazide therapy in a child with Pendred syndrome. *Clin Nephrol.* 2008;69(6):450-3.

12. Xu J, Song P, Nakamura S, Miller M, Barone S, Alper SL, et al. Deletion of the chloride transporter slc26a7 causes distal renal tubular acidosis and impairs gastric acid secretion. *J Biol Chem*. 2009;284(43):29470-9.

13. Gaarder PI, Heier HE. A human autoantibody to renal collecting duct cells associated with thyroid and gastric autoimmunity and possibly renal tubular acidosis. *Clin Exp Immunol.* 1983;51(1):29-37.

Figure 1

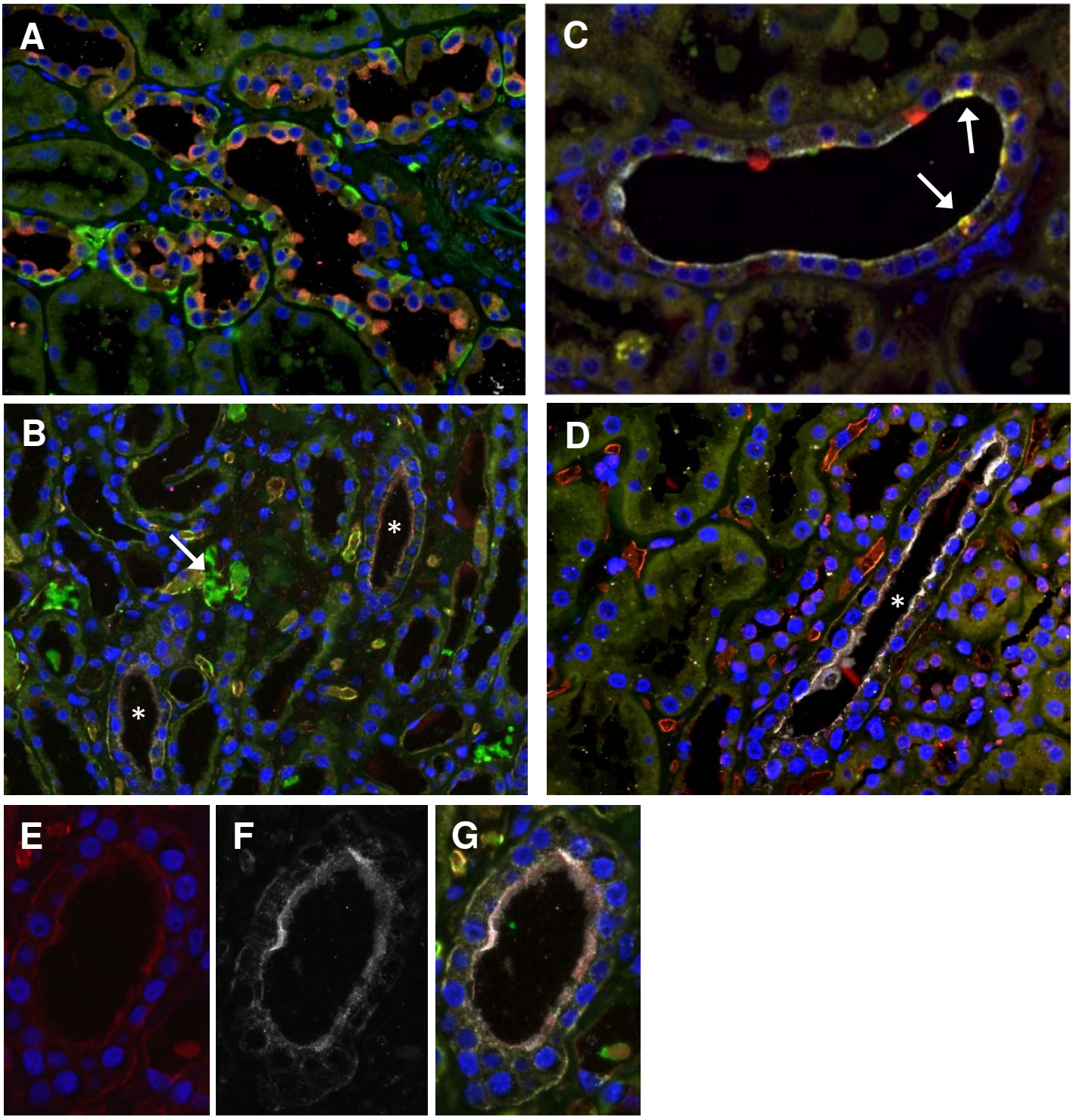

Figure 2